REMARKS

Reconsideration of this application is respectfully requested.

Claims 1-51 are presently pending in this application. Claims 1, 21 and 41 have been amended above to restore the claim language which was presented before Applicants filed their February 26, 1992 Amendment Under 37 C.F.R. §1.115. No claims have been added or cancelled by this Amendment. Accordingly, claims 1-51 are presented for further examination.

The Rejection Under 35 U.S.C. §102(b)/103

Claims 1 - 2, 4, 8, 12-14, 19 and 42 - 50 stand rejected under 35 U.S.C. §102(b) as anticipated by Miller et al. (Biochimie 67:769-776, 1985). In the Office Action (page 2), the Examiner stated:

> "Miller et al. discloses modified oligonucleotide compounds that fall within the definitions of the claimed compounds and a method for inhibiting the function of an RNA. Figure 4 on page 773 shows several specific oligonucleotides possessing methylphosphonate linkages that fully meet the applicant's claimed compounds.
>
> The applicant argues against this rejection on the basis that Miller et al. discloses exclusively oligomers in which all of the linkages are phosphonates and that these compounds fail to possess the additional criteria of the applicant that they create a RNAse sensitive duplexes with RNA. This argument is not deemed persuasive because the rejected claims are not limited by the functional language concerning the generation of an RNAse sensitive hybrid with RNA. Consequently, this rejection stands."

This ground of rejection is respectfully traversed.

The present invention is drawn to a modified nucleotide compound and to methods of identification and treatment. As disclosed in the instant specification (see page 4), the invention permits the rational design of stable therapeutically-effective oligo- and polynucleotides. More particularly, until the invention at hand, no one had examined the ability of mixed oligodeoxynucleotides to form RNase H-sensitive substrates as a factor in optimizing antisense function. Moreover, not until the invention at hand had anyone correlated the ability to form RNase

H sensitive substrate with nuclease resistance. Thus, there are inter alia two major features to the present invention: insensitivity to exo- and endonucleases, and the ability to form RNase H-sensitive hybrids. The latter feature plays a role in vivo the messenger RNA will be destroyed. There are cellular RNase H. If the antisense oligo is merely a roadblock to ribosomes, it binds to the RNA and blocks ribosome passage. Because there are RNase H in the cells, the antisense oligo can make the RNA hybrid into a target for RNase H, thereby helping to destroy the RNA rather than merely forming a roadblock to ribosome passage.

As stated in previous responses, Miller et al. discloses fully modified oligonucleotides. Having fully modified their oligonucleotide hybridized to RNA, Miller et al. proposes that the passage of ribosomes can be blocked. Applicants point out that RNA itself has loops and double-stranded regions which ribosomes have no trouble running through because the RNA gets translated. It is unlikely, therefore, that a small strand of oligonucleotides, such as disclosed by Miller et al., sufficiently blocks ribosomal passage given the fact that RNA is itself double-stranded in various regions. Reading takes place through such double-stranded areas, and the DNA-RNA hybrid that is formed is not as stable as the RNA-RNA hybrid that is formed by itself. Miller et al. does not even consider that a degradation of the RNA can be undertaken in their disclosure. They fully modify the oligonucleotides for protection from nucleases.

Applicants point out that all that is required for sufficient nuclease stability in a nucleotide compound is to modify the ends. In contrast to Miller et al., Applicants unexpectedly discovered that if one modifies at the ends and also has sufficient spacing of the modifications, one can achieve the nuclease stability reported by Miller et al. plus reap the benefits of forming an RNase H-sensitive hybrid, as discussed above. Thus, the instantly claimed modified nucleotide compounds do not act as mere roadblocks to ribosome passage, as disclosed in Miller et al.; instead, the instant modifications in effect serve a "catalytic-type" function, enhancing the sensitivity to RNase H when hybridized to RNA.

An additional feature is achieved by the instant invention over Miller et al. The modified nucleotide compound can cycle from one molecule or polymer of RNA to another because the RNA becomes destroyed by the RNase H. Further, the RNA may become destroyed even before it leaves the nucleus. Miller et al. does not disclose or even suggest that the RNA can be destroyed because of enhanced RNase H sensitivity; they merely disclose that the passage of ribosomes can be blocked by fully modifying oligodeoxyribonucleosides with methylphosphonate internucleotide bonds.

As far as a basis for rendering the instant invention obvious, Applicants respectfully point out that nowhere is there any mention or suggestion in Miller et al. that any modification other than full modification should be implemented for their disclosed oligodeoxyribonucleoside methylphosphonate compounds.

In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the rejection under §102(b), or alternatively under §103, based on Miller et al.

The Rejection Under 35 U.S.C. §102(b)

Claims 1-4, 12-14 and 42 - 50 stand rejected under 35 U.S.C. §102(b) as being anticipated by Stein et al. (Nucl. Acids. Res. 16(8):3209-3221 (1988)). In the Office Action (pages 2 and 3), the Examiner stated:

> "Stein et al. discloses modified oligomers with phosphorothioate linkages (see S-ODN-4 in Table 3, page 3216; this is an oligomer with phosphorothioate internucleotide linkages). Such oligomers are resistant to nuclease digestion and were able to inhibit the functioning of RNA by creating RNase sensitive duplexes (page 3220, last paragraph). The applicant argues that these modified oligomers containing phosphorothioate linkages do not anticipate the claimed compounds because they are not capable of hybridizing to a target RNA and that no mention is made in the reference of the sensitivity of the RNA-DNA duplex to RNAse. This argument has been fully considered but is not deemed persuasive because 1) the rejected claims are not limited to target RNA nor RNAse sensitive duplexes and 2) Stein et al. specifically notes that the RNA-DNA hybrids in which the DNA possesses phosphorothioate linkages are more sensitive to RNAse digestion than regular RNA-DNA duplexes (page 3320, last paragraph)."

The anticipation rejection is respectfully traversed.

Stein et al. report on the synthesis, melting temperatures and nuclease susceptibilities of a series of phosphorothioate oligodeoxynucleotide (ODN) analogs. In contrast to the present invention, Stein et al. do not disclose or suggest that an RNA molecule or polymer with A, C, G and/or U, will be sensitive when hybridized to a known sequence of phosphorothioate oligodeoxynucleotides. They only show hybridization to form duplexes of poly-rA/s-dT oligomers. Furthermore,

the inhibition of HIV activity by phosphorothioate-modified oligodeoxyribonucleotides may not be due to an antisense mechanism at all; instead, the anti-HIV activity reported by Stein et al. may be just as likely due to inhibition of reverse transcriptase, and other effects, as opposed to antisense function, as set forth in the instant invention. Thus, by using Stein's phosphorothioate oligodeoxyribonucleotides for an antisense purpose, one may actually be effecting other deleterious effects on cellular function, including DNA and RNA polymerase. Moreover, Stein's phosphorothioate oligodeoxyribonucleotides are all fully modified, in contrast to the instant "mixed" modified nucleotide compounds. As noted previously, Stein et al. only report or work with a synthetic RNA, which is far different from natural RNA. See the discussion in this Amendment above at page 3, first full paragraph, *supra*, relating to the first prior art rejection (Miller et al.).

In light of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the anticipation rejection.

The Rejection Under 35 U.S.C. §103

Claims 1-51 stand rejected under 35 U.S.C. §103 as being unpatentable over Walder et al. (PNAS 85: 5011, 1988) in view of Miller et al. (U.S. Patent No. 4,469,863) and Inoue et al. (Nucl. Acids Symposium Series 18: 958-976 (1988)).

In the Office Action (pages 3-5), the Examiner stated:

> "Walder et al. discloses that the most important element in the efficacy of antisense oligomers inhibiting mRNA expression is the formation of a RNAse sensitive RNA-DNA duplex that is cleaved by the enzyme: 'An important corollary of our results is that such modified analogs must not only retain normal hybridization properties but should also form substrates that are recognized and cleaved by RNAse H" (page 5015, second column, second paragraph).
>
> Miller et al. discloses antisense oligomers with all methylphosphonate internucleotide linkages. These modified oligomers possess resistance to nucleases, can pass through the membranes of mammalian cells, and can form stable duplexes with complementary mRNA (page 769, 'Summary').
>
> Inoue et al. teaches that a span as small as three contiguous phosphodiester linkages flanked by modified nucleotides (2'-O-methyl) was capable of forming an RNAse H-sensitive substrate (page 222, first paragraph)."

The Examiner stated further:

> "The claimed modified oligonucleotides posses three primary characteristics: 1) endo- and exonuclease resistance, 2) ability to hybridize to its RNA complementary sequence, and 3) the ability to form a RNAse sensitive RNA-DNA duplex.
>
> The person of ordinary skill in the art with the above references before him would have found the claimed modified oligomers obvious because of the necessity to have reduced the number of methylphosphonate internucleotide bonds in the oligomer in order to make the RNA-DNA duplex RNAse sensitive as Walder et al. emphasizes is critical to the efficacy of antisense oligonucleotides in inhibiting the [expression] of mRNA.
>
> The claimed methods of inhibiting the function of an RNA by contacting said RNA with a nuclease resistant antisense oligomer that forms RNAse H sensitive duplexes with said RNA would also have been obvious in view of the above references that, as a whole, teach the same method.
>
> Finally, the method for identifying modified antisense oligomers possessing the combination of nuclease resistance and the ability to form an RNAse H substrate with complexes of RNA using gel electrophoresis instead of the release of acid soluble radioactivity as taught by Walder et al. (page 5012, 'RNAse H Assay') would also have been obvious to the person of ordinary skill in the art. The use of gel electrophoresis is a fundamental tool in molecular biology for separating different types of polynucleotides whether by size or by other physical properties such as single-stranded versus double-stranded forms, linear versus circular forms, etc.
>
> The applicant's basic invention is the antisense oligomer with only a portion of the internucleotide linkages or bases modified in order to make the oligomer nuclease resistant. However, the prior art clearly teaches the necessity of combining both nuclease resistance with the ability to form RNAse H sensitive duplexes with RNA. The applicant's gel assay is only one way to assay for RNAse H sensitivity as Walder et al. substantiates."

The obviousness rejection is respectfully traversed.

In response to the rejection, Applicants wish to point out that the cited documents are so diverse in their disclosures that they are not properly combinable. On the one hand, Miller et al. discloses that oligonucleoside methylphosphonates act as a roadblock for ribosomal function, but the modified compounds do not cause any destruction in the RNA, albeit blocked from the ribosome. In effect, Miller et al. end up with a "bag" of RNA whose translation is blocked. To achieve that result, Miller et al. disclose that all of the oligodeoxyribonucleosides must be fully

modified with methylphosphonates. In Walder et al., all of their disclosure is directed to an *in vitro* system - having nothing to do with cells. In effect, Walder et al. discloses that there can be RNase H activity in the absence of any modification of the oligonucleotides. Walder et al. show that RNA can be broken up into smaller pieces *in vitro*. Walder et al. perform *in vitro* protein synthesis, demonstrating that if RNase H is present and if an oligodeoxyribonucleotide is hybridized to RNA, the RNA becomes cleaved and protein synthesis is inhibited. Significantly, Walder et al. are working with components in a tube without cells or DNase, and the antisense oligodeoxynucleotides are completely unmodified. With such oligodeoxynucleotides, the only effective way to inhibit translation is to degrade the RNA with an RNase H. Thus, Walder et al. proposes a protein-synthesizing system that includes RNase H and has no effect unless an unmodified oligodeoxynucleotides is present as well, that makes the RNA into an RNase H substrate. Hence, no protein synthesis is carried out. Thus Walder's disclosure is not concerned with a ribosome roadblock, as disclosed in Miller et al. Instead, Walder et al. disclose fully unmodified oligodeoxynucleotides which act as a portion of a substrate to inhibit protein synthesis.

The third cited document, Inoue et al., essentially shows that one can probe RNA with a structure that is a partially modified RNA sequence and partially a deoxynucleotide sequence; in other words, an oligoribodeoxynucleotide. Inoue's ribonucleotides are modified because RNA is so sensitive to RNase. Therefore, there are 2'-O-methyl groups on the RNA portion of their sequence, then a DNA stretch followed by another modified RNA stretch. In essence, for their disclosed oligonucleotides, Inoue et al. disclose RNA at the end which is modified and resistant to RNase H because of the modification on the 2' hydroxyl group which inhibits the RNase H. Inoue et al. do not disclose or suggest that such modifications or the modified oligonucleotide splints containing such modifications are resistant to DNase. In fact, Inoue et al. do not even take antisense function or activity into account, i.e., that the RNA has been cleaved to inhibit protein synthesis. Inoue et al. are employing their oligonucleotide splints as a molecular probe to study structure - to cleave specifically a particular RNA. Inoue et al. form a double strand that is recognized specifically and only by RNase H, much in the same way that a restriction enzyme recognizes and cuts specific nucleotide sequences. There is absolutely no contemplation by Inoue et al. to employ their modified oligonucleotide splints as antisense oligos for inhibiting protein synthesis.

Quite plainly, the combined cited disclosures are insufficient to have rendered the instant invention obvious at the time it was made. Reconsideration and withdrawal of the obviousness rejection is respectfully requested.

The Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 1 -51 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In the Office Action (page 6), the Examiner stated:

> "The critical issue in this rejection is that the applicant is attempting to define his invention primarily with functional language. This is inappropriate because the state of art of nucleic acid chemistry is well developed and thus allows compounds to be defined in specific structural terms. Without such specificity, it is practically impossible for the examiner to search the claims and equally difficult for the person of ordinary skill in the art to understand the metes and bounds of the invention.
>
> The applicant's arguments against each of the rejections under 35 U.S.C. §112, second paragraph, amounts to his stating that the law does not require greater specificity and that the claims are clear in view of the specifications. The examiner holds the opposite point of view for the reasons already of record on pages 5 - 8 of the first Office action on the merits mailed August 26, 1991 (Paper No. 8)."

In response to the rejection for indefiniteness, Applicants offer the following remarks.

It is respectfully maintained that the instantly claimed modified nucleotide compound (and the compound recited in the method claims) are well-defined in terms of its structure. The functional language that is recited in the instant claims is only employed to describe the characteristic(s) of the instant compound when the latter is modified in accordance with the instant invention.

In addition, Applicants reiterate by incorporating by reference the remarks presented on pages 15-21 of their February 26, 1992 in regard to the indefiniteness rejection. Reconsideration and withdrawal of the rejection is respectfully urged once again.

SUMMARY AND CONCLUSIONS

Claims 1-51 are presented for further examination. Claims 1, 21 and 41 have been amended; no claims have been added or cancelled by this Amendment. No new matter has been inserted by any of the foregoing changes to the instant claims which restores the original language.

This Amendment is accompanied by and includes a Request For a Three Month Extension of Time. The Patent and Trademark Office is hereby authorized to charge Deposit Account No. 05-1135 for the requisite fee of $420.00 for a small entity, as set forth in 37 C.F.R. §1.17(c). Small entity status for the instant assignee has been established by a concurrently filed declaration. The Patent and Trademark Office is further authorized to charge Deposit Account 05-1135 for any other fees in connection with this Amendment and to credit any overpayment thereto.

In view of the above discussion of the issues, Applicants respectfully submit that each of claims 1-51 is in condition for allowance. A favorable and speedy reconsideration of their rejection is requested. If any of these claims are found not to be in condition for allowance for any reason, the Examiner is respectfully requested to telephone the undersigned at (212) 924-5409 or 924-9578 to discuss the subject application.

Respectfully submitted,

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Date

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